A Comparison of Growth and Sucrose Metabolism in Sugarcane Germplasm from Louisiana and Hawaii

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ABSTRACT

Sugarcane (*Saccharum* sp. hybrids) genotypes bred in Hawaii are selected for maximum tonnage in a 2-yr production cycle and contain *S. robustum* germplasm. Genotypes bred in Louisiana are selected for high early sucrose yield after a 9-mo growing season, and contain *S. spontaneum* germplasm. We compared growth, sugar concentration, and enzymes of sucrose metabolism in four internodes of four Hawaii (HI) and two Louisiana (LA) genotypes during grand growth and ripening. On average, Sucrose concentrations were higher in LA than HI genotypes, especially at ripening. Fresh weight activities of soluble acid invertase (SAI) and neutral invertase (NI) were not different among genotypes at either growth stage. Significant differences in activities of sucrose synthase (SuSy), sucrose-phosphate synthase (SPS), and cell wall acid invertase (CWIN) among genotypes were not consistent between types. Sucrose concentration, total sugar concentration, and sucrose:total sugar ratio in the internodes were negatively correlated with water content, SAI activity, and NI activity, and positively correlated with the difference between SPS and SAI activity. These correlations seem to be a function of internode maturity. The consistent differences in sucrose content between LA and HI genotypes indicate the Louisiana and Hawaii breeding programs have produced very different genotypes, but these differences cannot be explained by differences in enzyme activities.

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Abbreviations: CWIN, cell wall acid invertase; FW, fresh weight; HI, Hawaii; LA, Louisiana; NI, neutral invertase; SAI, soluble acid invertase; SPS, sucrose-phosphate synthase; SuSy, sucrose synthase.

Commercial sugarcane cultivars are complex interspecific hybrids of up to five species of *Saccharum*: *S. officinarum, S. spontaneum, S. sinense, S. barberi,* and *S. robustum*. The breeding programs of Hawaii and Louisiana have different goals and use different germplasm. The breeding program in tropical Hawaii selects primarily for vegetative yield in an 18- to 24-mo production cycle. It uses little *S. spontaneum* germplasm. The Louisiana breeding program focuses on high, early sucrose yield in a 9-mo growing season (Breaux, 1984; Breaux, 1987). The Louisiana program uses *S. spontaneum* germplasm to increase ratooning (regrowth) ability and cold tolerance in this tropical crop, but no *S. robustum* is used since it cannot be made to flower in the short-growing season of Louisiana. Plant breeders in Louisiana are interested in using Hawaiian germplasm to expand the genetic base of sugarcane in Louisiana, hypothesizing that the alternate germplasm sources used by Hawaiian sugarcane breeders would bring new genes for biomass and sucrose into the Louisiana cultivar development program.

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Many of the classic experiments on sugarcane physiology and biochemistry were done in tropical environments such as Puerto Rico (Alexander, 1965; Alexander and Samuels, 1968), Australia (Glasziou and Bull, 1965; Glasziou and Gayler, 1972), and Hawaii (Goldner et al., 1991; Su et al., 1992). Most of these studies showed an inverse relationship between activity of the enzyme soluble acid invertase (SAI; EC 3.2.1.26), which cleaves sucrose into glucose and fructose, and sucrose accumulation in the stalk. Zhu and others (Zhu et al., 1997; Zhu et al., 2000) showed that the difference between the sucrose synthetic activity of sucrose-phosphate synthase (SPS; EC 2.4.1.14) and SAI activity was directly related to sucrose concentration in very diverse genotypes. In contrast, experiments done in a more temperate environment (Texas) using locally adapted commercial genotypes did not entirely support this relationship (Lingle, 1999). Rather, these studies showed that the difference between SPS and SAI was highly correlated with the sucrose to total sugar ratio, while sucrose content was correlated with SPS activity and inversely correlated with the water content of the internode. A recent study by Grof et al. (2007) showed a correlation between SPS activity in the upper internodes and whole-stalk sucrose yield in backcross progeny from a *S. officinarum* × commercial cultivar cross. This also highlights the role of SPS in sucrose accumulation in sugarcane.

The purpose of this study was to compare some aspects of growth and sucrose metabolism in internodes of germplasm from Louisiana and Hawaii. The goal was two-fold, to determine what effect bringing Hawaiian germplasm into the Louisiana sugarcane breeding program might have, and to determine if observed differences between experiments done in Hawaii and temperate climates such as Louisiana and Texas have been due to genotype or environment.

MATERIALS AND METHODS

Because of quarantine limitations, importation of adapted Hawaii cultivars into Louisiana was not possible in a timely manner. Therefore, we used true seed from polycrosses made in Hawaii, obtained from Dr. K.K. Wu, Hawaiian Agricultural Research Center, Aiea, HI, to establish four genotypes with Hawaiian (HI) germplasm. Genotypes 'US 02–101' and 'US 02–102' are progeny of the Hawaiian cultivar H78-3567 × elite Hawaiian genotypes, while 'US 02–103' and 'US 02–104' are progeny of 'H78-0292' × elite Hawaiian genotypes. Louisiana (LA) cultivars were represented by 'HoCP 85-845' and 'LCP 85-384,' which are products of five cycles of recurrent selection for sucrose in Louisiana.

Plant material for this experiment was sampled from single-row plots, 4.9 m long and 1.8 m apart, that were established by planting whole stalks in October 2001 at the USDA-ARS Ardoyne Research Farm near Schriever, LA (29°38′15″ N, 90°50′21″ W). The soil was Cancienne silt loam and Cancienne silty clay loam (fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquepts). Plots were fertilized with 112 kg ha^{-1} N, 15 kg ha^{-1} P, and 55 kg ha^{-1} K in the spring of each year. Stalks were sampled from the plant-cane crop in 2002 and the first regrowth (first-ratoon) crop in 2003.

As the sugarcane crop develops, it goes through several major stages in which the growth rate is altered. Following an initial lag period in the spring, the crop enters a period of rapid growth (grand growth), during which most of the biomass is accumulated. Later in the year, growth slows and the sucrose concentration in the juice increases (ripening). We sampled stalks at grand growth and ripening. The grand growth sampling dates were 9 July 2002 (plant-cane) and 25 July 2003 (first-ratoon), while the ripening sampling dates were 10 Oct. 2002 and 5 Nov. 2003. At each sampling date, we randomly removed four stalks from each genotype by cutting the stalks at ground level. Internode 1 was defined as that immediately below the point of attachment of the leaf with the topmost exposed dewlap (collar). We determined stalk length from the base of the stalk to the topmost exposed dewlap, above-ground internode number, and the length of internodes 2, 5, 8, and 11 from the top. Those internodes were chopped, frozen in dry ice, and stored at −80°C. The tissue was later crushed in liquid N_2 in a freezer mill (model 6800, SPEX CertiPrep, Metuchen, NJ), and again stored at −80°C. Enzyme extraction and assays and sugar concentrations were determined as previously described by Lingle (2004). Water content was determined by weighing remaining tissue before and after freeze-drying.

Procedures in 2003 were identical to those in 2002, except that rather than using the crude enzyme extract to also determine sugar concentrations, sugar was extracted from crushed tissue by refluxing 1 g fresh weight (FW) of crushed tissue twice in 3 mL water at 80°C, filtering the residue through filter paper, rinsing with another 3 mL of water, and combining the extracts. Sugars were separated using a CarboPac PA20 guard column (3 × 30 mm) and analytical column (3 × 150 mm) (Dionex Corp., Sunnyvale, CA) with 53 mM KOH as the eluent. Glucose, fructose and sucrose were detected with an ED50A integrated pulsed amperometric detector (Dionex), and quantified by comparison with external standards.

The data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 1998). The large effect of growth stage overwhelmed smaller differences between genotypes, so data were analyzed by stage. Internode was treated as a repeated measure. Because the two years also represent two crop ages (plant-cane and first-ratoon), year was treated as a fixed rather than a random effect. For variables for which the variances were proportional with the treatment mean squares (enzyme activities and sucrose concentration), data were transformed using the logarithmic transformation (Steel and Torrie, 1960) before analysis. Differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

The 2002 growing season was warmer and longer than the 2003 season (Fig. 1). Growing degree days were calculated by averaging the daily high and low temperatures, then subtracting the base temperature of 18°C (Bacchi and Sousa, 1977; Liu et al., 1998). The thermal clock was reset to 0°C-d when the low temperature was less than 0°C. In 2002 the grand growth and ripening samples were taken at 779 and 1669°C-d,

respectively, while in 2003, the samples were taken at 850°C-d and 1551°C-d.

Differences in stalk length were significant among genotypes during both grand growth and ripening (Table 1). Stalks of LA genotypes were significantly shorter than those of HI genotypes, mainly because one HI genotype, US 02–104, was about 40 cm taller than the next tallest genotype at grand growth and 73 cm taller at ripening. US 02–104 also grew more rapidly than the other genotypes between the sampling periods; it was 133 cm taller at ripening than at grand growth. In contrast, another HI genotype, US 02–103, grew only 90 cm between the two sampling times. This was less than the growth of the two LA genotypes. Therefore, there were no consistent differences in growth rate between the two groups of genotypes. Since the HI genotypes were unselected progeny, it was expected that there would be variation among them in stalk length and growth rate.

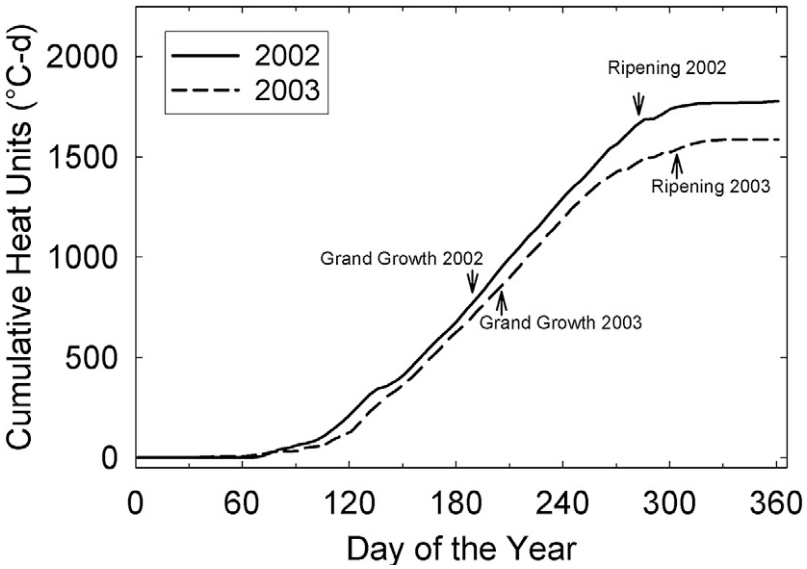

Figure 1. Accumulation of heat units with a base temperature of 18°C at Ardoyne Research Farm near Schriever, LA in 2002 and 2003. Arrows indicate when stalk samples were taken each year.

There were no significant differences among genotypes in the number of above-ground internodes at the first sampling during grand growth (Table 1), but there were significant differences at ripening. US 02–104 had the most internodes at ripening (27.0), and produced the most (13.5) between grand growth and ripening (Table 1). US 02–103 had the fewest internodes at ripening (18.4) and produced the fewest during the study period (6.5). The differences between genotype groups were not significant.

The effect of year on both stalk length and internode number was significant only for the grand growth sampling (Table 1). The grand growth sampling in 2003 was 16 d or 71°C-d later than that in 2002. Plots were sampled later in 2003 because in the first sampling in 2002 some of the sampled stalks did not yet have 11 internodes. The growth rate at grand growth in the two years was very similar: 0.21 cm °C-d^{-1} in 2002, and 0.22 cm °C-d^{-1} in 2003. The rate of internode production at grand growth was also very similar in both years.

The length of selected internodes, while different among genotypes at grand growth (Table 2), was not different between groups at either stage. At grand growth, LCP 85–384 and US 02–104 had longer internodes than the other genotypes. Differences in internode length among genotypes were not significant at ripening. Internodes 2, 5, and 8 were shorter at ripening than at grand growth. This is reflective of the slower growth rate that accompanies ripening in sugarcane, as previously observed (Lingle, 1997; Lingle, 1999).

Internode water content was significantly different among genotypes and between groups at grand growth and ripening (Table 2). Internodes of the two LA genotypes had lower water contents than all the HI genotypes except US 02–103 at both growth stages. The water content of the individual internodes decreased with internode age, and was lower, on average, at ripening than at grand growth (Fig. 2). This reduction in water content is also indicative of ripening (Lingle, 1999). At ripening, the

Table 1. Effect of year and genotype on mean stalk length and internode number of sugarcane genotypes adapted to Louisiana or Hawaii, sampled at grand growth and ripening in 2002 and 2003.

	Stalk length		Internode no.	
	Grand growth	Ripening	Grand growth	Ripening
	cm			
Year				
2002	163.5b†	292.9a	11.1b	20.5b
2003	187.1a	277.9a	13.4a	22.8a
Genotype				
HoCP 85-845	154.6c	264.4b	11.9a	21.8b
LCP 85-384	172.5b	278.2b	11.5a	21.3bc
US 02-101	156.3c	275.7b	12.0a	21.5bc
US 02-102	169.5b	272.0b	12.6a	20.0bc
US 02-103	180.8b	271.1b	11.9a	18.4c
US 02-104	218.0a	351.0a	13.5a	27.0a
State				
LA	163.6b	271.3b	11.7a	21.5a
HI	181.1a	292.4a	12.5a	21.7a
			P > F	
Year	***	NS‡	***	NS
Genotype	***	***	NS	***
LA vs. HI	**	*	NS	NS

*Significant at $P \le 0.05$.

**Significant at $P \le 0.01$.

***Significant at $P \le 0.001$.

†Means within a group (year, genotype or state) followed by the same letter are not significantly different by the protected LSD test at $P \le 0.05$ ($n = 4$).

‡NS, not significant.

Table 2. Effect of year, genotype (G), and internode (I) position on mean length, water content, total sugar concentration, sucrose concentration, and sucrose: total sugar ratio of internodes of sugarcane genotypes adapted to Louisiana (LA) or Hawaii (HI), sampled at grand growth and ripening in 2002 and 2003.

	Internode length		Water content		Total sugar concentration		Sucrose concentration		Sucrose:Total sugar ratio	
	Grand growth	Ripening	Grand growth	Ripening	Grand growth	Ripening	Grand growth	Ripening	Grand growth	Ripening
	cm		kg kg^{-1} FW†		mmol kg^{-1} FW					
Year										
2002	10.9a‡	10.1a	0.876a	0.848a	273.9a	366.2a	64.2a	122.1a	0.298a	0.440b
2003	11.2a	7.9b	0.862b	0.812b	216.8b	250.7b	46.1b	124.4a	0.315a	0.587a
Genotype										
HoCP 85-845	10.2cd	8.8a	0.850b	0.807b	266.0ab	351.2ab	88.5a	189.6a	0.439a	0.661a
LCP 85-384	12.0ab	8.9a	0.866ab	0.810b	288.6a	363.4a	72.6a	174.6a	0.347ab	0.593a
US 02-101	9.7d	8.8a	0.882a	0.849a	244.7bc	309.2b	48.3bc	100.4b	0.276bc	0.440b
US 02-102	10.1d	9.2a	0.877a	0.856a	219.4cd	231.5c	38.5cd	81.0b	0.226c	0.377b
US 02-103	11.3bc	10.5a	0.859b	0.804b	253.1abc	317.5b	65.4ab	164.8a	0.330bc	0.603a
US 02-104	13.0a	7.8a	0.880a	0.856a	199.9d	248.0c	33.1d	78.9b	0.222c	0.408b
State										
LA	11.2a	8.8a	0.856b	0.808b	278.7a	357.3a	82.7a	183.5a	0.393a	0.627a
HI	11.0a	9.0a	0.874a	0.841a	229.3b	284.1b	46.4b	102.9b	0.263b	0.457b
Internode										
2	6.4c	4.7c	0.927a	0.913a	147.5c	162.8c	19.6c	35.2d	0.155c	0.285d
5	13.1a	8.4b	0.898b	0.849b	203.8b	281.5b	22.2c	94.2c	0.118d	0.385c
8	13.6a	10.8a	0.844c	0.804c	298.9a	381.0a	109.6b	235.7b	0.391b	0.641b
11	11.2b	12.1a	0.805d	0.765d	331.1a	408.5a	184.2a	294.8a	0.562a	0.743a
						***P* > F**				
Year	NS§	***	***	***	***	***	***	NS	NS	***
G	**	NS	*	**	**	***	**	***	***	***
LA vs. HI	NS	NS	*	**	***	***	***	***	***	***
I	***	***	***	***	***	***	***	***	***	***
G×I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

***Significant at $P \leq 0.001$.

†FW, fresh weight.

‡Means within a group (year, cultivar, state or internode) followed by the same letter are not significantly different by the protected LSD test at $P \leq 0.05$ ($n = 4$).

§NS, not significant.

water contents of internode 2 of the two LA genotypes and US 02–103 were somewhat less than that of the other HI genotypes, and the decrease in water content between internodes 2 and 4 was greater in the LA genotypes and US 02–103, suggesting that internodes of these genotypes matured faster than those in the other genotypes.

There were significant differences among genotypes and groups in the average total sugar concentration at grand growth and ripening (Table 2). At grand growth, the average total sugar concentration ranged from 200 mmol kg^{-1} FW in US 02–104 to 289 mmol kg^{-1} FW in LCP 85–384. At ripening, the mean total sugar concentration of all genotypes had increased. US 02–104 still had the lowest concentration, 248 mmol kg^{-1} FW, and LCP 85–384 had the highest, 363 mmol kg^{-1} FW. LA genotypes had significantly higher total concentration than HI genotypes at both stages, although the concentration in US 02–103 approached that of the LA genotypes. As expected, total sugar increased with internode age, and between grand growth and ripening. At grand growth, the increase in total sugar with internode down the stalk was faster in the LA than HI genotypes (Fig. 3A). This could be because less sugar was translocated to internodes of the HI than the LA genotypes, or the HI genotypes were utilizing more sugar for other processes, such as cell wall biosynthesis or respiration. At ripening, the total sugar accumulation curves of all the genotypes were roughly parallel (Fig. 3B), especially after internode 5. Internodes 5, 8, and 11 of the LA genotypes had consistently higher total sugar concentrations than the HI genotypes.

Not only did the LA genotypes accumulate higher total sugar concentrations than the HI genotypes, but more of that sugar was sucrose. Sucrose concentration varied significantly among genotypes and groups (Table 2). Among genotypes,

sucrose showed a similar trend as total sugar. US 02–104 had the lowest mean sucrose concentration of all genotypes at both grand growth and ripening. HoCP 85–845 had the highest concentration. The differences in the sucrose concentration among the different internodes among genotypes were notable. During grand growth, little sucrose accumulated in internode 2 or 5 (Fig. 4A). In internodes 8 and 11, the sucrose concentration of the LA genotypes was greater than in the other genotypes, although in internode 11, the sucrose concentrations of US01–101 and US 02–103 approached those of the LA genotypes. During ripening, there was a distinct difference in sucrose accumulation pattern. The sucrose concentration of internode 2 was similar in all genotypes (Fig. 4b). Sucrose concentration in internode 5 of HoCP 85–845, LCP 85–384, and US 02–103 was markedly greater than in internode 2, but this was not true of the other HI genotypes. At ripening, the sucrose concentration of internode 11 of Louisiana genotype HoCP 85–845 was 426 mmol kg^{-1} FW, more than twice that of US 02–102, 197 mmol kg^{-1} FW.

Ripening in sugarcane is defined as an increase in sucrose yield per Mg ha^{-1} of harvested cane (Legendre, 1975). This increase is accomplished by an increase in total sugar and an increase in juice purity, defined as the percentage of soluble solids in juice that is sucrose. The soluble solids in juice are predominantly sugars, and therefore the sucrose:total sugar ratio is one measure of harvest maturity. Differences among genotypes and groups in the sucrose:total sugar ratio were significant (Table 2). The LA genotypes had a higher mean ratio at grand growth and ripening. In internodes 2 and 5 at grand growth, the ratio was similar in all genotypes, except HoCP 85–845, which had a higher ratio than the others in internode 2 (Fig. 5a). In internodes 8 and 11 there was a much wider range of ratios. The two LA genotypes had the highest ratios and the HI genotypes the lowest, except for US 02–103, which had the same ratio in internode 11 as LCP 85–384. At ripening, the genotypes could be easily divided into two groups on the basis of the sucrose:total sugar ratio in internodes 5, 8, and 11 (Fig. 5b). HoCP 85–845, LCP 85–384, and US 02–103 had higher ratios than the other genotypes.

Differences among genotypes in activities of SAI and neutral invertase (NI) were not significant at either growth stage (Table 3). In the case of SAI, the differences between the HI and LA genotypes were significant at $P \leq 0.05$ during both grand growth and ripening. At grand growth, the LA genotypes had higher mean SAI activity, while at ripening, the HI genotypes had higher mean SAI activity. However, in each case the differences were due to one genotype in the group that had especially high mean SAI activity rather than each of the genotypes of one group having higher SAI activity than the other group.

Sucrose synthase (SuSy) activity was not significantly different among genotypes at grand growth, and although there were significant differences in SuSy activity at

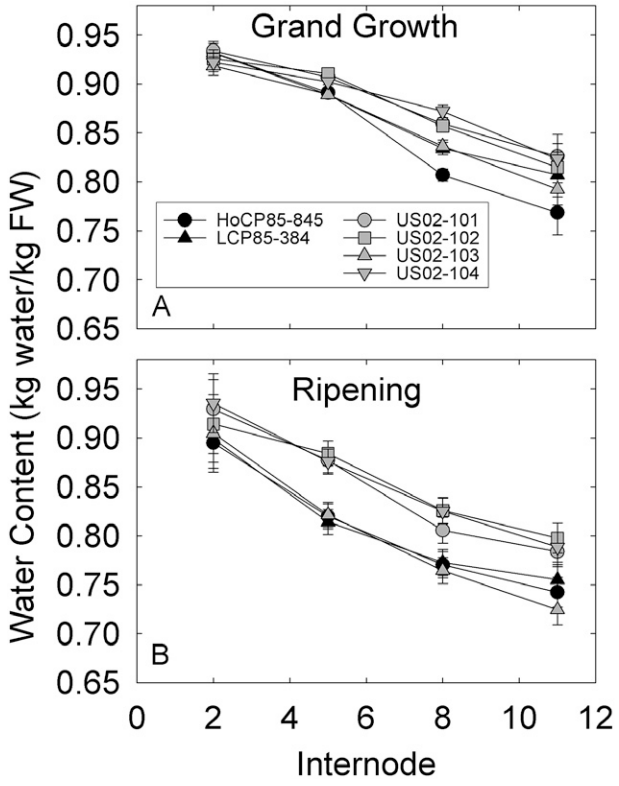

Figure 2. Water content of sugarcane internodes, counted down from the topmost visible collar, at grand growth and ripening. Black symbols denote Louisianan genotypes, gray symbols Hawaiian genotypes. Vertical bars represent ± SE (n = 8).

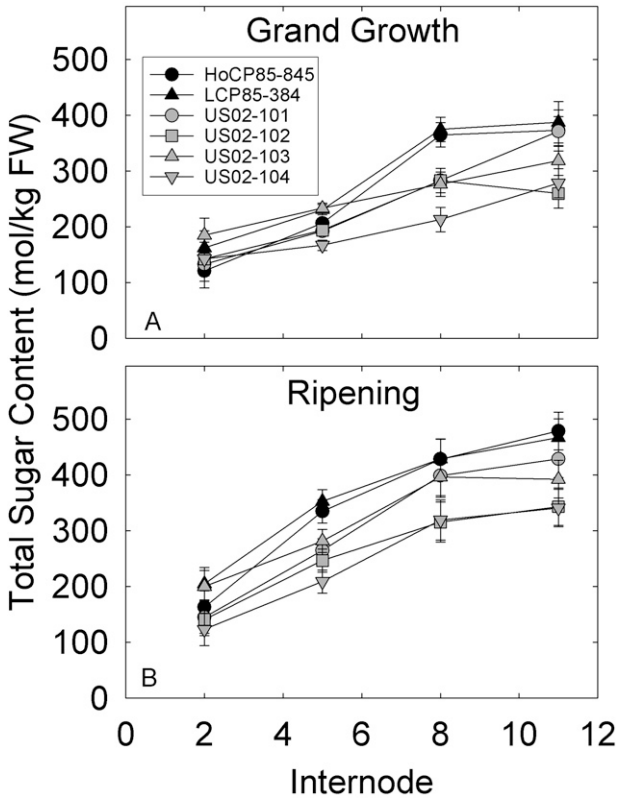

Figure 3. Total sugar concentration of sugarcane internodes counted down from the topmost visible collar, at grand growth and ripening. Black symbols denote Louisianan genotypes, gray symbols Hawaiian genotypes. Vertical bars represent ± SE (n = 8).

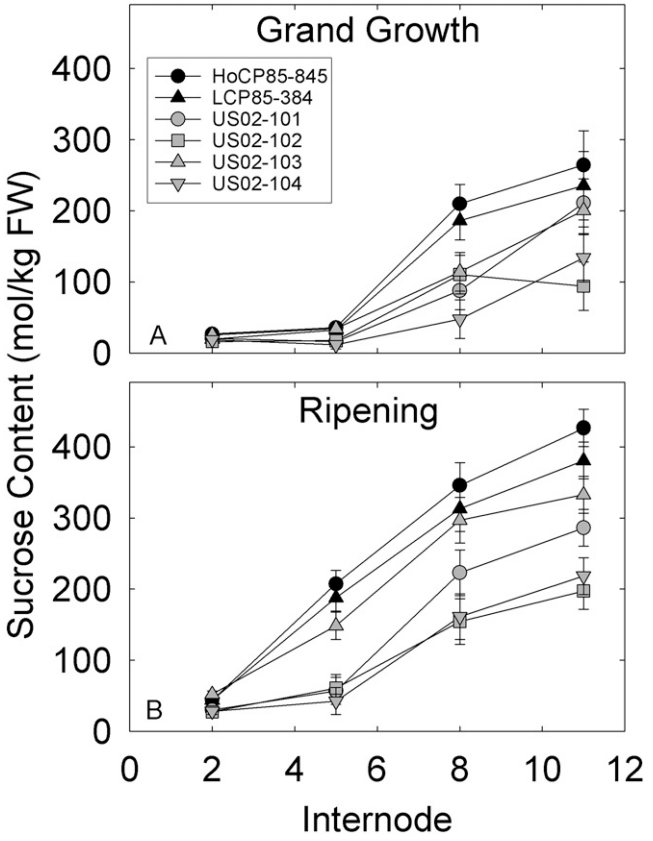

Figure 4. Sucrose content of sugarcane internodes counted down from the topmost visible collar, at grand growth and ripening. Black symbols denote Louisianan genotypes, gray symbols Hawaiian genotypes. Vertical bars represent ± SE (n = 8).

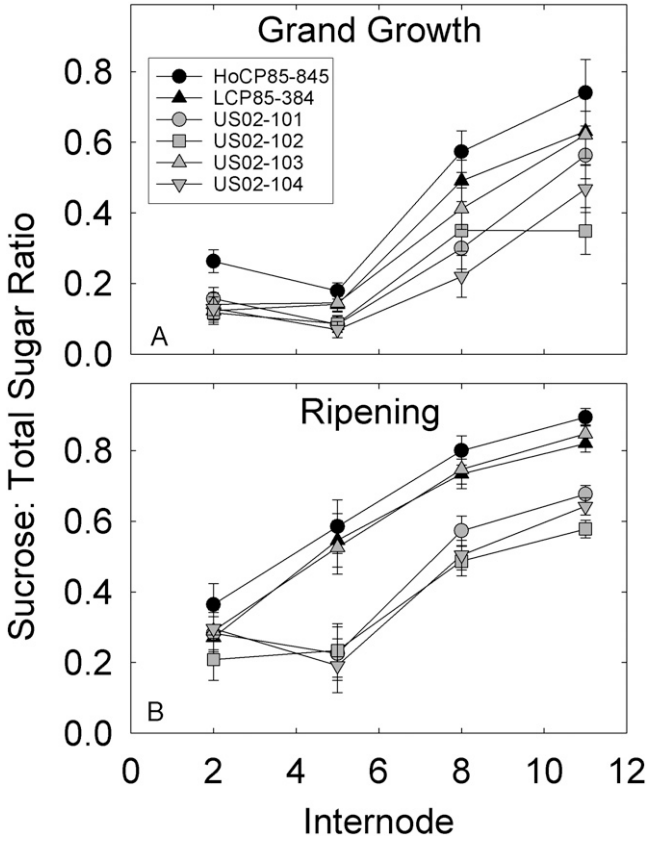

Figure 5. The sucrose:total sugar ratio of sugarcane internodes counted down from the topmost visible collar, at grand growth and ripening. Black symbols denote Louisianan genotypes, gray symbols Hawaiian genotypes. Vertical bars represent ± SE (n = 8).

ripening, these differences were not significant between groups. There were significant differences in SPS activity among genotypes at both growth stages (Table 3). At grand growth the differences between groups was significant, but the differences were not consistent between groups. Two of the HI genotypes, US 02–101 and US 02–102, had activities similar to LCP 85–384 and HoCP 85–845, respectively, while US 02–103 and US 02–104 had lower activities. Differences between genotype groups for SPS activity during ripening were not significant between groups.

At grand growth, the difference between genotype groups in mean cell wall acid invertase (CWIN) activity was consistent (Table 3). Cell wall acid invertase activity was significantly higher in all four HI genotypes than in the two LA genotypes, and differences among genotypes and among genotype groups were highly significant at $P \leq 0.001$. The differences between groups had disappeared at the ripening stage.

The activity of all enzymes except CWIN was highest in the youngest internode, and decreased in older internodes. This has been observed numerous times (Lingle, 1997; Zhu et al., 1997). Since enzyme activity is expressed on a FW basis, the decrease with age is primarily caused by the expansion of the cells of the internode. There were differences in the degree of decrease among the enzymes. For instance, SAI during grand growth decreased from 20.22 mmol kg^{-1} FW h^{-1} in internode 2 to 1.28 mmol kg^{-1} FW h^{-1} in internode 11, while SuSy decreased from 8.31 mmol kg^{-1} FW h^{-1} to 6.49 mmol kg^{-1} FW h^{-1}. CWIN activity increased with internode during grand growth, from 1.79 mmol kg^{-1} FW h^{-1} in internode 2 to 2.66 mmol kg^{-1} FW h^{-1} in internode 11. This tendency for CWIN activity to be higher in older internodes has also been noted before (Vorster and Botha, 1999; Lingle, 2004).

Simple correlations among water, SAI, NI, SuSy, SPS, CWIN, and SPS-SAI with sucrose, total sugar, and the sucrose:total sugar ratio indicated that water content and the two soluble invertases were highly negatively correlated with sucrose, total sugar and the sucrose:total sugar ratio (Table 4). The high negative correlation of water content with total sugar ($r = -0.871$, $P < 0.01$), sucrose ($r = -0.931$, $P < 0.01$) and the sucrose:total sugar ratio ($r = -0.955$, $P < 0.01$) is similar to that observed in earlier experiments (Lingle, 1999). The correlation of water content with total sugar may be partly due to a displacement effect; i.e., as dry matter (sugar) increases with internode development, water content decreases. However, displacement does not explain the decrease in water content and increase in total sugar, sucrose, and sucrose:total sugar ratio between grand growth and ripening (Fig. 2, 3, and

Table 3. Effect of year, genotype (G), and internode (I) position on mean fresh weight (FW) activity of soluble acid invertase, neutral invertase, sucrose synthase, sucrose-phosphate synthase, and cell wall acid invertase of sugarcane genotypes adapted to Louisiana (LA) or Hawaii (HI), sampled at grand growth and ripening in 2002 and 2003.

	Soluble acid invertase		Neutral invertase		Sucrose synthase		Sucrose-P synthase		Cell wall acid invertase	
	Grand growth	Ripening	Grand growth	Ripening	Grand growth	Ripening	Grand growth	Ripening	Grand growth	Ripening
	mmol sucrose cleaved or synthesized kg^{-1} FW h^{-1}									
Year										
2002	6.82a†	1.71a	0.81a	0.61a	8.29a	8.77a	1.60a	1.40a	2.01b	3.17a
2003	5.49b	1.59a	0.93a	0.58a	5.78b	4.58b	1.63a	1.24a	2.57a	2.09b
Genotype										
HoCP 85-845	7.72a	1.79a	0.73a	0.51a	6.50a	6.67ab	2.45a	1.26cd	1.87cd	2.36bc
LCP 85–384	5.76a	1.07a	0.76a	0.58a	6.31a	5.91ab	1.44b	1.47abc	1.03d	3.55a
US 02-101	5.35a	1.28a	0.88a	0.75a	6.55a	8.60a	1.56b	1.91a	2.42bc	3.08ab
US 02-102	5.83a	1.76a	1.13a	0.49a	6.39a	4.86b	2.20a	1.62ab	2.33bc	2.06c
US 02-103	5.22a	1.36a	0.75a	0.61a	9.16a	5.92ab	1.28bc	1.05d	2.76ab	2.61bc
US 02-104	7.05a	2.65a	0.95a	0.65a	7.28a	8.10ab	0.79c	0.62d	3.31a	2.14c
State										
LA	6.72a	1.43b	0.75a	0.55a	6.47a	6.29a	1.94a	1.36a	1.45b	2.95a
HI	5.86b	1.76a	0.93a	0.62a	7.35a	6.87a	1.46b	1.30a	2.71a	2.47a
Internode										
2	20.22a	5.13a	2.11a	1.09a	8.31a	9.22ab	3.63a	3.12a	1.79cd	2.24a
5	2.09b	0.64b	0.59b	0.56b	6.72b	6.94a	1.15b	0.86b	2.02bc	3.05a
8	1.03c	0.44bc	0.41b	0.35c	6.61b	5.87b	0.71c	0.65b	2.69a	2.76a
11	1.28bc	0.40c	0.36b	0.39bc	6.49a	4.67c	0.98bc	0.66b	2.66ab	2.48a
						P > F				
Year	**	NS§	NS	NS	***	***	NS	NS	*	***
G	NS	NS	NS	NS	NS	*	***	*	***	*
LA vs. HI	*	*	NS	NS	NS	NS	*	NS	***	NS
I	***	***	***	***	*	***	***	**	*	NS
G×I	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*Significant at $P \leq 0.05$.

**Significant at $P \leq 0.01$.

***Significant at $P \leq 0.001$.

†Means within a group (year, cultivar, state, or internode) followed by the same letter are not significantly different by the protected LSD test at $P \leq 0.05$ ($n = 4$).

§NS, not significant.

4). Withholding water can be used in some areas to ripen sugarcane before harvest (Ludlow et al., 1992; Robertson and Donaldson, 1998; Yates, 1996). This specific effect may be mediated by the actions of SAI. In this study and others (Lingle, 1997; Lingle 1999), water content and SAI activity declined with ripening, and glyphosate used as a chemical ripener decreases the activity of SAI in elongating internodes (Zhu et al., 1997).

The negative correlations of SAI and NI with sucrose, total sugar, and the sucrose:total sugar ratio (Table 4) indicate that these enzymes have a strong influence on these parameters of sugar concentration, but the lack of differences between LA and HI genotypes in enzyme activities indicate that the correlations of SAI and NI with the sugars are a function of internode development, not a function of genotype group. The activities of SuSy, SPS, and CWIN were not significantly correlated with sucrose, total sugar, or the sucrose:total sugar ratio, suggesting that these enzymes do not have a direct influence on sugar storage in sugarcane internodes. The difference between SPS and SAI activities was positively correlated with sucrose, total sugar, and the sucrose:total sugar ratio. This result is similar to that reported by Zhu et al. (1997), who showed that the average sucrose concentration of highly diverse sugarcane genotypes was positively correlated to the difference between SPS and SAI activities. Although SPS activity alone was not correlated with sugar concentration, the difference between SPS activity and SAI activity explained more of the variation in the data than with SAI alone. Results from Zhu et al. (1997), and unpublished results from this laboratory, demonstrated a threshold effect: Sucrose does not accumulate in internodes with greater than a certain amount of SAI activity, but once SAI activity in an internode is less than that threshold, sucrose concentration is more influenced by SPS activity.

Table 4. Pearson correlation coefficients of mean sucrose concentration, total sugar concentration, and sucrose:total sugar ratio with water content, the fresh weight activities of soluble acid invertase (SAI), neutral invertase (NI), sucrose synthase (SuSy), sucrose-phosphate synthase (SPS), cell wall acid invertase (CWIN), and the difference in activities of SPS and SAI (SPS-SAI), among six sugarcane genotypes across 2 yr and four internodes (n = 12).

	Sucrose	Total sugar	Ratio
Water	–0.931**	–0.871**	–0.955**
SAI	–0.689*	–0.709**	–0.704*
NI	–0.734**	–0.702*	–0.788**
SuSy	NS†	NS	NS
SPS	NS	NS	NS
CWIN	NS	NS	NS
SPS-SAI	0.697*	0.738**	0.704*

*Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

†NS Not significant.

CONCLUSIONS

As a group, the HI genotypes were significantly different from LA genotypes in sugar accumulation. The LA genotypes accumulated higher concentrations of total sugar and sucrose in their internodes than the HI genotypes (Table 2), although one of the HI genotypes accumulated almost as much sucrose and had the same sucrose:total sugar ratio as the LA genotypes. Because the HI genotypes were unselected progeny from elite parents in Hawaii, and not tested cultivars, some variation is expected. The fact that three of the four had distinctly different sugar accumulation patterns suggests that the different germplasm sources used in HI and LA, and the different selection goals in the two programs, have resulted in genotypes with different sucrose partitioning strategies. The differences in partitioning of sucrose among competing sinks between and within internodes were not due to differences in stalk growth rate, since there was no difference in growth rate between the LA and three of the HI genotypes (Table 1). There may have been differences in stalk diameter or fiber content, which we did not measure. There may also have been differences in photosynthetic rate, but this also was not measured. Bringing Hawaiian germplasm into the Louisiana sugarcane breeding program will expand the genetic base of new genotypes, but may also result in decreases in sucrose content and delays in ripening compared to genotypes resulting from traditional germplasm sources. Extra effort will be needed to overcome these disadvantages.

While a significant negative correlation between sucrose concentration and SAI and NI activities indicate these enzymes may play a role in sucrose accumulation in sugarcane internodes, differences in sugar accumulation between HI and LA genotypes could not be explained by differences in the activities of enzymes of sucrose metabolism between the two groups. Also, differences in the relationships between enzymes and sucrose accumulation between experiments done in Hawaii and Louisiana are more likely to be due to environmental rather than genotypic differences.

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References

Alexander, A.G. 1965. Physiological studies of enzymes catalyzing the synthesis and hydrolysis of sucrose, starch and phosphorylated hexose in sugargane. J. Agric. Univ. Puerto Rico 49:60–75.

Alexander, A.G., and G. Samuels. 1968. Controlled-temperature studies of growth, enzymology, and sucrose production by two sugarcane varieties in Puerto Rico. J. Agric. Univ. Puerto Rico 52:204–217.

Bacchi, O.O.S., and J.A.G.C. Sousa. 1977. Minimum threshold temperature for sugarcane growth. Proc. Int. Soc. Sugar Cane Technol. 16:1733–1742.

Breaux, R.D. 1984. Breeding to enhance sucrose content of sugarcane varieties in Louisiana. Field Crops Res. 9:59–67.

Breaux, R.D. 1987. Some breeding strategies with bi-parental and polycrosses. p. 71–85. *In* Copersucar Int. Sugarcane Breeding Workshop, Piracicaba-SP, Brazil. May–June 1987. Cooperative of Producers of Cane, Sugar and Alcohol of the State of Sao Paulo, Piracicaba-SP, Brazil.

Glasziou, K.T., and T.A. Bull. 1965. The relation between total invertase activity and internode expansion in sugarcane stalks. Proc. Int. Soc. Sugar Cane Technol. 12:575–581.

Glasziou, K.T., and K.R. Gayler. 1972. Storage of sugars in stalks of sugar cane. Bot. Rev. 38:471–490.

Goldner, W., M. Thom, and A. Maretzki. 1991. Sucrose metabolism in sugarcane cell suspension cultures. Plant Sci. 73:143–147.

Grof, C.P.L., P.L. Albertson, J. Bursle, J.M. Perroux, G.D. Bonnett, and J.M. Manners. 2007. Sucrose-phosphate synthase, a biochemical marker of high sucrose accumulation in sugarcane. Crop Sci. 47:1530–1539.

Legendre, B.L. 1975. Ripening of sugarcane: Effects of sunlight, temperature, and rainfall. Crop Sci. 15:349–352.

Lingle, S.E. 1997. Seasonal internode development and sugar metabolism in sugarcane. Crop Sci. 37:1222–1227.

Lingle, S.E. 1999. Sugar metabolism during growth and development in sugarcane internodes. Crop Sci. 39:480–486.

Lingle, S.E. 2004. Effect of transient temperature change on sucrose metabolism in sugarcane internodes. J. Am. Soc. Sugar Cane Technol. 24:132–141.

Liu, D.L., G. Kingston, and T.X. Bull. 1998. A new technique for determining the thermal parameters of phenological development in sugarcane, including suboptimum and supra-optimum temperature regimes. Agric. For. Meteor. 90:119–139.

Ludlow, M.M., R.C. Muchow, and G. Kingston. 1992. Quantification of the environmental and nutritional effects on sugar accumulation. p. 27–35. *In* J.R. Wilson (ed.) Improvement of yield in sugar cane through increased sucrose accumulation. CSIRO, Brisbane, Australia.

Robertson, M.J., and R.A. Donaldson. 1998. Changes in the components of cane and sucrose yield in response to drying-off of sugarcane before harvest. Field Crops Res. 55:201–208.

SAS Institute. 1998. The SAS system for Windows. Release 7.0. SAS Inst., Cary, NC.

Steel, R.G.D., and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill, New York.

Su, L.Y., A.D. Cruz, P.H. Moore, and A. Maretzki. 1992. The relationship of glyphosate treatment to sugar metabolism in sugarcane: New physiological insights. J. Plant Physiol. 140:168–173.

Vorster, D.J., and F.C. Botha. 1999. Sugarcane internodal invertases and tissue maturity. J. Plant Physiol. 155:470–476.

Yates, R.A. 1996. Factors that affect the quality of sugarcane: Part 1. Climate. Int. Sugar J. 98:280–284.

Zhu, Y.J., H.H. Albert, and P.H. Moore. 2000. Differential expression of soluble acid invertase genes in the shoots of high-sucrose and low-sucrose species of *Saccharum* and their hybrids. Aust. J. Plant Physiol. 27:193–199.

Zhu, Y.J., E. Komor, and P.H. Moore. 1997. Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. Plant Physiol. 115:609–616.